

NOTE ON CYTOKININS IN THE XYLEM SAP OF *PROTEA COMPACTA*

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ABSTRACT

Partition chromatography on Sephadex LH-20 indicated that zeatin is probably the major cytokinin present in the transpiration stream of *Protea compacta* seedlings.

UITTREKSEL

NOTA AANGAANDE SITOKINIENE IN DIE XILEEMSAP VAN *PROTEA COMPACTA*

Met behulp van partisie-chromatografie op Sephadex LH-20 is gevind dat zeatin waarskynlik die belangrikste sitokiniene in die transpirasiestroom van *Protea compacta* saailinge is.

A number of reports have indicated that endogenous cytokinins are involved in the growth and development of proteaceous species (Brown and Van Staden, 1973; Van Staden and Brown, 1973). As very little information is available as to the nature of the cytokinins involved an attempt was made to learn more about the cytokinin complement of *Protea compacta* R.Br.

To simplify investigation the xylem sap from six month old seedlings was analyzed. Xylem sap was assayed as it is well known that the roots of plants act as a site of cytokinin synthesis and that these substances are translocated in the transpiration stream (Kende and Sitton, 1967; Yoshida *et al.*, 1971). In addition xylem sap does not contain many impurities which necessitate complicated purification techniques.

Plants growing in pots were decapitated 2 cm above soil level. Root exudate was collected for 24 hours by means of a rubber tube attached to the stem. The sap was collected, filtered and taken to dryness under vacuum at 35°C. The residue was washed with 10 ml 80% ethanol, filtered, concentrated to 2 ml and separated on a Sephadex LH-20 column (2.5 x 90 cm). The column was eluted with 35% ethanol at a flow rate of 15 ml/hour. This technique of fractionation has been shown to be a reliable and reproducible method of cytokinin separation (Armstrong *et al.*, 1969). It is also most useful for the identification of cytokinins in plant material (Van Staden and Drewes, 1975). Forty ml fractions were collected from the column. These were dried in air and assayed for cytokinin activity with the soybean bioassay (Miller, 1965).

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Three peaks of activity, that co-eluted with authentic cytokinins, were recorded in the root exudate (Fig. 1). The results indicate that zeatin appears to be the major translocation form of cytokinin in the xylem sap of *P. compacta* seedlings. However, small amounts of zeatin riboside and 6-(Δ^2 -isopentenylumino) purine (2iP) were also apparently present. The finding that zeatin is probably the major cytokinin in the xylem sap of *P. compacta* is contrary to Letham's (1974) suggestion that zeatin riboside is the major form in which cytokinin is translocated from the roots of plants. It is however noticeable that in all experiments, where zeatin riboside predominated, the root exudate was partitioned against butanol prior to fractionation. Although it does not form part of the present investigation, evidence is available which indicates that zeatin is not totally recovered when plant extracts are partitioned against butanol.

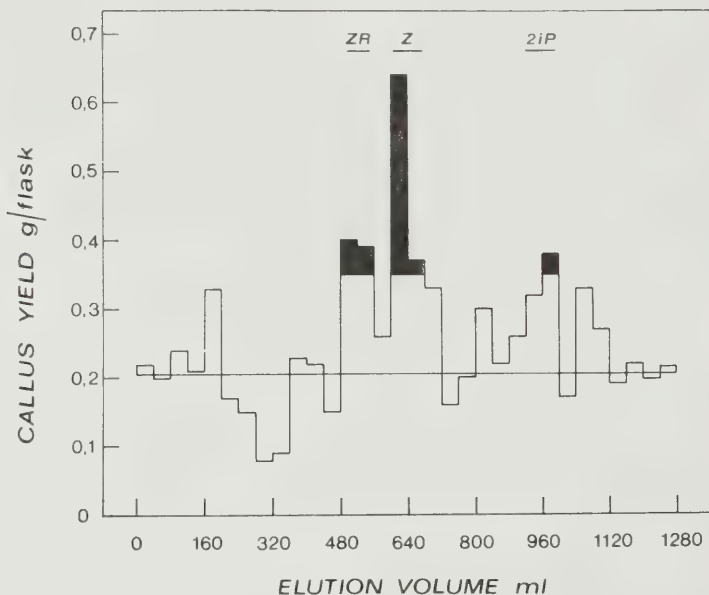


FIG. 1.

Soybean bioassay of 50 ml of root exudate of *Protea compacta* fractionated on Sephadex LH-20.

Z = zeatin, ZR = zeatin riboside, 2iP = 6-(Δ^2 -isopentenylamino) purine.

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